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14. ABSTRACT
We are proposing to study CDCA7L in an NF1 mutant model of astrocytoma and glioblastoma and neurotransmitter levels in NF1 mutant brains, comparing males and females. The results of this work can be used to develop additional hypotheses on whether a "yin-yang" relationship exists in males and females between risk for brain cancer and risk for depression, or other learning and social dysfunctions. Developing new treatments for gliomas and learning/social dysfunction through a better understanding of the basic biology will benefit both male and female NF1 patients in the long term.

15. SUBJECT TERMS
Nothing listed

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1. Introduction

CDCA7L encodes the R1 transcription factor that is known to cooperate with Myc in transcriptional regulation, as well as repress the expression of *MAOA* and *MAOB* that are important regulators of catecholamines in the brain. We have shown that *CDCA7L* stimulated the growth of male astrocytic tumor cells and astrocytes, but has little to no effect on female astrocytic tumor cells and astrocytes. Males are at a greater risk for brain tumors and females are at a greater risk for depression that can be linked to changes in catacholamine levels. We have therefore hypothesized that *CDCA7L* may have different molecular propensities in males and females and are examining the role of Chr Y-specific epigenetic modifiers in affecting *CDCA7L* mode of action. NF1 patients are at a greater risk for both astrocytoma/glioblastoma and depression, so we are particularly interested in whether *NF1* mutations affect the molecular function of *CDCA7L* and whether sex-specific treatments may be more effective for treating these complications of NF1. The goal of this project is to better understand regulation of *CDCA7L* expression, to characterize a new mouse mutant of *Cdca7l*, to look at the effect of *Cdca7l* loss on *Nf1* tumorigenesis in male and female mice, and to examine the effect of *Cdca7l* loss on brain development. astrocyte phenotypes, and catecholamine levels.

2. Keywords

Neurofibromatosis type 1
CDCA7L
astrocytoma
glioblastoma
MAO
catecholamines
sex differences
mouse models

3. Accomplishments

Major Goals and Accomplishments:
In the previous year, we accomplished the following according to the task laid out in the SOW:

1) Establish maintenance colonies for C57BL/6N-*Cdca7l*-/+, C57BL/6J-*Nf1*-/+;*Trp53*-/+*cis*, C57BL/6J-*Nf1*-/+, and C57BL/6J-*beta-actin-Cre-ERT* for breeders needed in Task 2, 3, 4, and 5.

We continue to maintain colonies of mutant mice as described in the Statement of Work. Because the original mutation in *Cdca7l* (Fig 1B) was found to be a hypomorph, we crossed the mice to ACT-FLPe mice to generate a floxed allele (Fig 1C), and crossed to CMV-Cre mice to generate an allele missing exons 5 to 9, expected to be a null allele (Fig 1D). We have confirmed the rearrangement of the null allele (Fig 1D) using PCR primers within the construct. Our efforts to confirm that there is no expression from the null allele have given us uninterpretable results thus far, possibly due to overlapping gene transcripts in the region. We continue to test new primer sets and antibodies to resolve this issue. We are currently sequencing the genomic DNA through the region to confirm the rearrangement is as we expect.

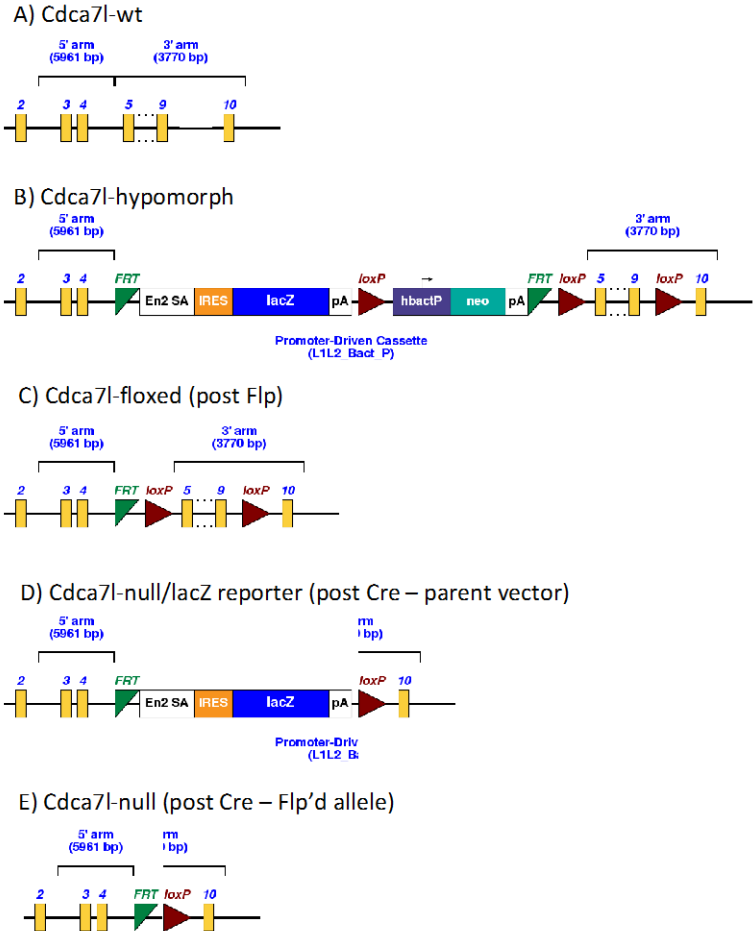

Figure 1: Allele configurations for *Cdca7l* mutant mice.

2) Establish expression pattern of *Cdca7l* in males and females during brain development using

a LacZ insertion reporter. (Months 3-6)

We began this task using the hypomorph allele in the previous reporting period, and will complete this task, once the sequencing described under Task 1 is completed.

3) Determine null and heterozygous *Cdca7l* mutant phenotype. (Months 3-21)

We have intercrossed the *Cdca7l* mice heterozygous for the null allele (Fig 1D) and have gotten null/null mice, suggesting the mutation is not embryonic lethal. In crosses of *Cdca7lnull/+* X *Cdca7lnull/+* 50% of progeny were *Cdca7lnull/+,* 29% of progeny were wild type, and 21% of progreny were *Cdca7lnull/null.* We have established an aging cohort of 10 males and 10 females each for *Cdca7l-null/null* and *Cdca7l-null/wt* along with wild-type littermates and are aging these to look for any obvious differences in lifespan or behavior. The oldest of these mice is currently 6.5 months old and no obvious phenotypes have been observed yet. We also euthanized 3 males and 3 females each for *Cdca7l-null/null* and *Cdca7l-null/wt* along with wild-type littermates at 9 weeks of age and surveyed all organs. No clear differences have been detected yet and we are still awaiting detailed review by our veterinary pathologist. Because we have not detected a postnatal phenotype yet, we will wait to collect the prenatal timepoints until the postnatal timepoints are thoroughly analyzed.

4) Test the hypothesis that reduced levels of *Cdca7l* inhibit brain tumors in *NPcis* mice (Months 7-34)

We are beginning to intercross the *Cdca7l-null* mice with the *NPcis* mice. We expect to finish these crosses in the next few months, and then age progeny up to 1 year. In the past year we have tested whether stable knockdown of *CDCA7L* in human glioblastoma cell lines inhibit growth *in vivo* by subcutaneous injection into athymic mice. Knockdown of *CDCA7L* in male U118MG cells completely blocked growth *in vivo* (Fig 2A), whereas knockdown in female U87MG cells led to much faster growth (Fig 2B).

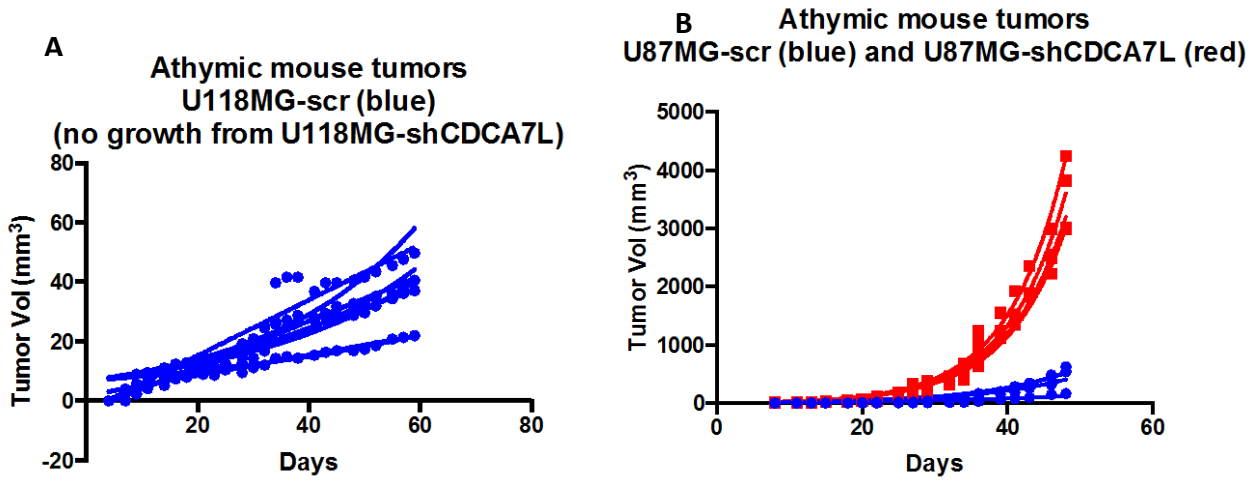

Figure 2: Growth of individual tumors injected subcutaneously in athymic mice. Growth of cells stably transfected with a scramble shRNA vector control (blue) or an shRNA-CDCA7L knockdown construct (red). (A) male U118MG cells. (B) female U87MG cells.

5) Identify overlap of SNPs with putative *CDCA7L* regulatory regions. (Month 1-2)

This task was mostly completed in the previous reporting period. Given the increasing availablility of human ENCODE data on transcription factor binding sites, we are reanalyzing the sites we originally identified. We expect to complete this task this summer.

6) Determine haplotypes of existing BAC libraries and human cell lines. (Months 3-4)

Due to advances in CRISPR technology, we are planning to test whether direct mutation of transcription factor binding sites in cell lines and looking at *CDCA7L* expression is a better approach than engineering BAC luciferase reporters. We will be conducting pilots this summer to decide whether to

switch our approach or keep the approach as described in Tasks 6-7.

7) Engineer and test BAC luciferase reporters for *CDCA7L* regulation by the *CDCA7L-RAPGEF5* intergenic region. (Months 5-10)

See Task 6

8) Test candidate trans-acting regulatory factors in the control of *CDCA7L* expression. (Months 11- 15)

We will initiate this task once Tasks 6-7 are complete, or we have tested regulatory regions using CRISPR technology.

9) Examine the interaction of changing *Cdca7l* levels with *Nf1* mutation on brain phenotypes, catecholamine levels, and response to dopamine pathway therapeutics (months 3-9 and month 21-26)

We will collect this data as soon as the sequencing results are completed as described in Task 1.

Opportunities for Training and Professional Development:

This award has supported the training of one post-doctoral fellow, Dr. Min-Hyung Lee. During this reporting period, Dr. Lee presented his work at the Children's Tumor Foundation 2015 NF Research Symposium, giving him opportunities to learn from and network with leaders in the NF field. Dr. Lee moved to another position midway through this reporting period, so travel and training funds have been reserved for his replacement, Mr. Mackenzie Silverman (see (5) Changes/Problems below for more details).

In addition, Dr. Lee and I meet several times a week to go over experimental design. We are currently revising his manuscript on the role of *CDCA7L* in brain cancer, and I expect the incoming post-baccalaureate fellow, Mr. Silverman, to help complete the final experiments needed for resubmission.

Dissemination of Results:

Dr. Lee has presented his work on CDCA7L at the Children's Tumor Foundation 2015 NF Research Symposium as described under (6) Products. We are revising a manuscript based on his findings so far on the sex-specific activity of CDCA7L. As noted under Major Goals and Accomplishments, most of the major goals of the project are still in the early phases, in part due to changes in personnel this reporting period (see (5) Changes/Problems below for more details) and are not ready to be publically disseminated.

Plans for Next Reporting Period:

In the next reporting period we will finish characterization of the *Cdca7l-null* allele. If necessary, we will cross the *Cdca7l-flox* allele to Cre to get a true null allele (Fig 1E). We will generate *NPcis;Cdca7l* mutant mice for aging studies and generate *Nf1;Cdca7l* mutant mice to collect brains for catecholamine analysis.

We will test whether CRISPR can be used to mutate regulatory regions in human and mouse cells to look at changes in *CDCA7L* expression, and whether we can adequately quantify *CDCA7L* directly or whether cloning of the luciferase gene into the *CDCA7L* locus in necessary to measure expression levels. Depending on the results of these pilots, we will test regulatory regions either using CRISPR or BAC-luciferase reporters.

Depending on the results of the testing of regulatory regions, we will begin depletion of transcription factors that bind to the regulatory regions of interest.

We expect that we will need to request a no cost extension in the next reporting period.

4. Impact:

We have nothing new to report this period, beyond what was stated in the previous annual report.

5. Changes/Problems:

Dr, Min-Hyung Lee, the post-doctoral fellow dedicated to the project, left the laboratory November 1, 2015 to start a second post-doctoral fellowship with the FDA and pursue his interests in the regulatory field. Because of the Center for Cancer Research's policy of only hiring post-doctoral fellows for a minimum of 3 years, it was not possible to directly replace Dr. Lee. I have been working with the Geneva Foundation on different mechanisms to fill this position. I have recently hired a post-baccalauerate fellow, Mr. Mackenzie Silverman, for the upcoming year who will begin work in early June, 2016. Many of the molecular biology tasks have been on hold until the new fellow is in place. Due to this delay in hiring, plus the time it will take Mr. Silverman to get up to speed on the projects, I anticipate I will need to request a no cost extension. Because Mr. Silverman's salary and benefits are significantly less that Dr. Lee's, I am working to hire an additional post-baccalaureate fellow with complimentary experience to work with Mr. Silverman on the project.

While we have generated the mouse *Cdca7l-null* allele that was a difficulty during the last reporting period, testing the expression in these mice has led to results that are difficult to explain, and have caused us to resequence through the genomic region of the rearrangement. Some of the tasks related to these mice have been held back until we have these results. We have also generated the flox allele for *Cdca7l* and if the "null" allele has rearranged to give and unexpected result by sequencing, we will cross the floxed allele to Cre to generate an additional form of the null allele, although one that lacks the LacZ reporter gene (Fig 1E).

Due to advances in CRISPR technology in recent years, we will investigate whether directly mutating regulatory DNA sequences in cell lines and measuring *CDCA7L* expression directly is a better method for Tasks 6-7 than engineering BAC luciferase reporters. Depending on pilot experiments, we will either switch to CRISPR technology, or keep the original method as outlined in the statement of work. Mr. Silverman will begin this work when he joins the lab in June. If applicable, we will request prior approval prior to implementing any change to the Statement of Work.

6. Products:

Conference Papers and Presentations:

Poster Presenter: Dr. Min-Hyung Lee
Title: *CDCA7L* is a gender-specific modifier of astrocytoma and glioblastoma
Date: 6/7/16
Location: Children's Tumor Foundation 2015 NF Conference, Monterey, CA

Oral Presenter: Dr. Karlyne Reilly
Title: Sex bias in astrocytoma/glioblastoma: *CDCA7L* as a male-specific oncogene
Date: 4/18/16
Location: Understanding the Sex Bias in Disease Symposium, NIH, Bethesda, MD

Research Materials:
Cdca7l mutant mouse allelic series: hypomorph, floxed allele, null allele

7. Participants and Other Collaborating Organizations:

Name:	Min-Hyung Lee
Project Role:	Post-doctoral Fellow
Researcher Identifier:	
Nearest person months worked:	5
Contribution to the project:	Dr. Lee has been working on the mechanism of how CDCA7L functions in males and females. He tested the growth of glioblastoma cells with *CDCA7L* knockdown in athymic mice. Dr. Lee's salary is provided by this award. Dr. Lee left the project on November 1, 2015 to start a fellowship at the FDA.
Name:	Karlyne Reilly
Project Role:	Principal Investigator
Researcher Identifier:	0000-0001-9109-4409

Nearest person months worked:	3
Contribution to the project:	Dr. Reilly has monitored the mouse colony, determining which breeders to set-up and which mice to euthanize for analysis. She has also managed the budget and supervised Dr. Lee and Mr. Tuskan. Dr. Reilly's salary is provided by the National Cancer Institute.
Name:	Robert Tuskan
Project Role:	Technician
Researcher Identifier:	
Nearest person months worked:	2
Contribution to the project:	Mr. Tuskan has prepared tail DNA from the *Cdca7l* mouse colony and genotyped them for whether they carry the *Cdca7l* mutation. Mr. Tuskan is designing primers to sequence the *Cdca7l* allele construct. Mr. Tuskan also helps to monitor the budget and places all orders for the grant. Mr. Tuskan's salary is provided by the National Cancer Institute.
Organization Name:	Leidos
Location:	Frederick, MD
Project Role:	Animal Technical Support and Histology Technical Support
Nearest person months worked:	2
Contribution to the project:	The animal technical support staff at NCI, Frederick monitor the health of the mouse colony, set up breeders, tail clip mice, and euthanize mice under Dr. Reilly's instruction. The histology technical support at NCI, Frederick, euthanize and dissect mice as needed for the project and process tissues for histology. Slides are sent to Dr. Reilly for review.

Changes in active support: Nothing to Report.